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Genetic Factors of Uterine Hyperplastic Diseases.

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ABSTRACT

The article highlights the bioinformatics analysis data of five polymorphous loci among 947 patients with uterine hyperplasia and 988 women from the control group. It was found that the increased risk of uterine hyperplasia in women of Russia Central region is connected with the combination of alleles C rs12444979 with G rs2241423 (OR = 1.57), and the protective effect have the combinations of the following molecular genetic markers: C rs12444979 with A rs999460 with G rs2241423 with G rs6732220 (OR = 0.67), A rs999460 with a G rs2241423 with G rs6732220 (OR = 0.69), C rs12444979 with A rs999460 with G rs6732220 (OR = 0.71) and A rs999460 with G rs6732220 (OR = 0.72).

Keywords: uterine hyperplasia, genetic polymorphism, bioinformatics.

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INTRODUCTION

Uterine hyperplastic processes (UHP) (uterine myoma, genital endometriosis, endometrial hyperplasia) take leading place in the structure of common gynecological diseases. They have common pathogenesis and therefore are combined quite often (Klatsky et al., 2008). The main clinical manifestations of these diseases are uterine bleeding, pelvic pain, infertility and miscarriage (Taran et al., 2014).

Uterine myoma is benign and usually multiple tumor, growing from immature myocytes of the vascular uterine wall (Churnosov et al, 2014;. Tan et al, 2014.). Endometriosis – is the pathological process, characterized by growth and development of tissues, identical in structure and function to the endometrium, outside the boundaries of normal localization of uterine body mucous membrane. (Truskinovsky et al, 2014.). Endometrial hyperplastic processes are interpreted as non-physiological proliferation of endometrial glands, which is accompanied by structural changes of glandular and, to a lesser extent, stromal components of the endometrium. (Donato et al, 2014;. Pachomov et al, 2014.).

It is believed that uterine myoma, genital endometriosis, hyperplastic processes of the endometrium are hormone-driven processes. The leading role in the pathogenesis of uterine hyperplastic processes is given to excess estrogen stimulation, combined with the lack of progesterone exposure (Kim et al., 2013). However, up to date, there is no common understanding of the development mechanisms of benign tumors in uterine tissues.

One of the factors, determining the development of uterine hyperplasia, may be the age of menarche and related with it candidate genes (Elks et al., 2010).

MATERIALS AND METHODS

The objects and methods of the research. The analysis of the surveillance results was carried out among 1935 people: 947 patients with uterine hyperplasia and 988 women from the control group. The samples of patients and control included women of Russian nationality, who are natives of the Central Black Soil Region of the Russian Federation and do not consist in relationships with each other. Clinical and instrumental examination of patients with hyperplastic processes of the uterus was carried out by doctors of gynecological department of the Perinatal Center of the Belgorod Regional Clinical Hospital of St. Joasaph. The control group included women with no gynecological problems.

All patients with uterine hyperplastic processes and the individuals in the control group had a typing of five molecular-genetic markers: CNVs g.19933600C> T (rs12444979), SFTA3 c.-2153G> A (rs999460), MAP2K5 c.1135-12146G> A (rs2241423) , FSHR c.375-5096C> G (rs6732220), LHCGR c.3441 + 42609C> T (rs4953616).

The venous blood in the amount of 8-9 ml, taken from the cubital vein of proband was the material for the study. The isolation of genomic DNA from peripheral blood was conducted by standard method of phenol-chloroform extraction (Miller et al., 1988). The analysis of studied loci was carried by the method of polymerase chain reaction of DNA synthesis, using oligonucleotide primers and probes.

The statistical processing of data was performed using the software packages «STATISTICA for Windows 6.0» and «Microsoft Excel 2007". The criterion χ^2 was used in order to analyze the compliance of observed distribution of genotypes with the expected, based on the Hardy-Weinberg equilibrium.

The analysis of the role of studied genes combinations in the formation of uterus hyperplastic processes was performed by applying the software APSampler, using Monte Carlo Markov chains and Bayesian nonparametric statistics (Favorov et al., 2005). In the process of conducting multiple comparisons, in order to minimize the errors of the first kind, the permutational test (p_{perm}) was used.

RESULTS AND DISCUSSION

The analysis of genes polymorphisms was performed on the material of two samples: 947 patients with uterine hyperplasia and 988 women from the control group. The samples of patients with uterine

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hyperplasia and control group included women of Russian nationality, who are natives of the Central Black Soil Region of the Russian Federation and do not consist in relationships with each other. Patients were included in the group of medical cases only after making the disease diagnosis, confirmed by clinical laboratory and by instrumental methods of examination. Clinical and laboratory examination of patients was carried out on the basis of gynecological department of the Perinatal Center of the Belgorod Regional Clinical Hospital of St. Joasaph.

The study of the alleles frequency of polymorphic genes markers revealed, that for all studied loci, in patients with uterine hyperplasia and in patients from the control group, the empirical distribution of genotypes corresponds theoretically expected, at the Hardy-Weinberg equilibrium (p> 0.05) (Table 1).

Table 1: Summary information about the studied polymorphisms

Polymorphism	Studied groups	Minor allele	MAF (%)	HWE	
				χ^2	p
GPRC5B g.19933600C>T (rs12444979)	Case	T	10.08	0.70	>0.05
GPRC5B g.19933600C>T (rs12444979)	Control	T	11.66	0.66	>0.05
SFTA3 g.9920G>A (rs999460)	Case	A	16.53	0.46	>0.05
SFTA3 g.9920G>A (rs999460)	Control	A	19.64	0.92	>0.05
MAP2K5 c.1243-12146G>A (rs2241423)	Case	A	14.48	0.79	>0.05
MAP2K5 c.1243-12146G>A (rs2241423)	Control	A	17.27	1.36	>0.05
FSHR c.375-5096C>G (rs6732220)	Case	G	16.70	0.04	>0.05
FSHR c.375-5096C>G (rs6732220)	Control	G	18.50	0.09	>0.05
LHCGR c.3441+42609C>T (rs4953616)	Case	C	49.80	1.70	>0.05
LHCGR c.3441+42609C>T (rs4953616)	Control	C	48.35	4.19	>0.05

Notes: MAF, minor allele frequency; Hardy – Weinberg equilibrium. P values were calculated using the χ^2 test.

As a result of bioinformatics analysis of carrier the alleles and genotypes combinations of investigated loci, the number of significant differences between patients with uterine hyperplasia and patients from the control group was identified (Table 2).

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Table 2: Prevalence of some combinations of the studied polymorphic markers in patients with uterine hyperplasia and patients from the control group

SNP 1	SNP 2	SNP 3	SNP 4	Carriage		Fisher's p-value (Bonferroni correction, p_{cor})	Odds ratio (95% CI)
				Case	Control		
C rs12444979	A rs999460	G rs2241423	G rs6732220	19.65	26.63	0.0002 ($1.6*10^{-6}$)	0.67 (0.54-0.84)
A rs999460	G rs2241423	G rs6732220		20.63	27.43	0.0004 ($6.3*10^{-6}$)	0.69 (0.55-0.85)
C rs12444979	A rs999460	G rs6732220		20.75	26.85	0.001 (0.0006)	0.71 (0.57-0.89)
A rs999460	G rs6732220			21.63	27.59	0.001 (0.005)	0.72 (0.58-0.89)
C rs12444979	G rs2241423			95.90	93.71	0.02 (0.05)	1.57 (1.03-2.40)

The highly significant (Pperm = 1.6 * 10-6) association of alleles combination C rs12444979 with A rs999460 with G rs2241423 and G rs6732220 with the formation of uterine hyperplastic processes, comes under notice first of all. This combination is occurred among 19.65% of patients with uterine hyperplastic processes, and in 26.63% of patients from the control group. This combination of polymorphic genes variants is the protective factor of uterine hyperplastic processes development, as evidenced by the value of OR, which is equal to 0.67 at the 95% confidence interval 0.54-0.84.

It was found that the combination of three genetic variants A rs999460 with G rs2241423 with G rs6732220 and C rs12444979 with A rs999460 with G rs6732220 were occurred among the patient from the control group (27.43% and 26.85%, respectively), that is in 1.29-1.33 times higher than among patients with uterine hyperplastic processes (20.63%, Pperm=$6.3*10^{-6}$ and 20.75%, Pperm = 0.0006, respectively). With these combinations of polymorphic markers, the risk of development of uterine hyperplasia is decreased (OR = 0.69 and OR = 0.71, respectively). The differences of similar orientation are recorded for the combination of two genetic variants A rs999460 with G rs6732220. Among patients with uterine hyperplasia, it was recorded the lowest frequency of this combination (21.63%) compared to the control group (27.59%, Pperm = 0.005, OR = 0.72).

In addition, the association of alleles combinations C rs12444979 with G rs2241423 with the formation of uterine hyperplasia was identified: in 95.90% of patients with uterine hyperplasia, this combination of genetic markers was registered, and in the patient from the control group, this coefficient was 93.71% (Pperm = 0.05, OR = 1.57, 95%, CI 1.03-2.40).

As a result of bioinformatics analysis of alleles frequencies and genotypes of studied genes between the patients with uterine hyperplastic processes and the patients from control group, it was found, that the risk factor for the contraction of this pathology is a combination of alleles C rs12444979 with G rs2241423 (OR = 1.57).

A large number of literature shows the relationship of these genes with the formation of metabolic disorders (including obesity) (Mei et al, 2012; Rask-Andersen et al, 2012; Yang et al, 2013) and thus they are associated with the early age of menarche, and this may be a risk factor for the development of uterine hyperplastic processes (Demerath et al., 2013).

CONCLUSION

Thus, in the process of the research it was found, that the risk of uterine hyperplastic processes in women of the Central Black Soil Region of the Russian Federation, is raised by the combination of alleles C rs12444979 with G rs2241423 (OR = 1.57), and the combination of the following molecular genetic markers has protective properties: C rs12444979 with A rs999460 with G rs2241423 with G rs6732220 (OR = 0.67), A

rs999460 with G rs2241423 with G rs6732220 (OR = 0.69), C rs12444979 with A rs999460 with G rs6732220 (OR = 0.71) and A rs999460 with G rs6732220 (OR = 0.72).

REFERENCES

[1] Churnosov, M.I., Altuchova, O.B., Demakova, N.A., Krivoshei, I.V., Evdokimov V.I., Batlutskaya I.V., Polonikov A.V., 2014. *Associations of Cytokines Genetic Variants with Myomatous Knots Sizes*. // Research Journal of Pharmaceutical, Biological and Chemical, 5(6): Pp. 1344-1347.

[2] Demerath, E.W., Liu, C.-T., Franceschini, N., Chen, G., Palmer, J.R., Smith, E.N., Chen, C.T.L., Ambrosone, C.B., 2013*. Genome-wide association study of menarche age in African-American women.* // Hum Mol Genet, 22(16): Pp. 3329–3346.

[3] Donato, N.D., Seracchioli R., 2014. *How to Evaluate Adenomyosis in Patients Affected by Endometriosis?* Electronic source. Available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4146361/ (accessed 12.08.2014).

[4] Elks, C. E., Perry, J. R. B., Sulem, P., Chasman, D. I., Franceschini, N., He, C., Lunetta, K. L., 2010. *Thirty new loci for age at menarche identified by a meta-analysis of genome-wide association studies*. // Nat Genet, 42(12): Pp. 1077–1085.

[5] Favorov, A.V., Andreewski, T.V., Sudomoina, M.A., Favorova, O.O., Parmigiani, G., Ochs, M. F., 2005. *Markov chain Monte Carlo technique for identification of combinations of allelic variants, underlying complex diseases in humans.* // Genetics, 171(4): Pp. 2113-2121.

[6] Kim, J.J., Kurita, T., Bulun, S. E., 2013. *Progesterone Action in Endometrial Cancer, Endometriosis, Uterine Fibroids, and Breast Cancer.* // Endocrine Reviews, 34(1): Pp. 130–162.

[7] Klatsky, P.C., Tran, N.D., Caughey, A.B., Fujimoto, V.Y., 2008. *Fibroids and reproductive outcomes: a systematic literature review from conception to delivery. //* American journal of obstetrics and gynecology, 198 (4): Pp. 357-366.

[8] Mei, H., Chen, W., Jiang, F., He, J., Srinivasan, S., Smith, E.N., Schork, N., Murray, S., Berenson, G.S., 2012. *Longitudinal Replication Studies of GWAS Risk SNPs Influencing Body Mass Index over the Course of Childhood and Adulthood.* Electronic source. Available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3280302/ (accessed 12.02.2012).

[9] Miller, S.A., Dykes, D.D., Polesky, H.F., 1988. *A simple salting out procedure for extracting DNA from human nucleated cells. //* Nucleic acids research, 16(3): Pp. 1215-1221.

[10] Pachomov, C.P., Altuchova, O.B., Demakova, N.A, Krivoshei, I.V., Kolesnikov Y.V., Sobyanin F.I., 2014. *Study of Cytokines Polymorphous Loci Connections with Rise of Endometrium Proliferative Diseases.* // Research Journal of Pharmaceutical, Biological and Chemical, 5(6): Pp. 1473-1476.

[11] Rask-Andersen, M., Jacobsson, J. A., Moschonis, G., Ek, A.E., Chrousos, G.P., Marcus, C., Manios, Y., Fredriksson, R., Schiöth, H. B., 2012. *The MAP2K5-linked SNP rs2241423 is associated with BMI and obesity in two cohorts of Swedish and Greek.* // BMC Med Genet, 13: Pp. 36-39.

[12] Tan, N., 2014. *Women seeking second opinion for symptomatic uterine leiomyoma: role of comprehensive fibroid center.*//Journal of Ther Ultrasound, 2. Available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4265989/ (accessed 15.04.2014).

[13] Taran, F.A., Weaver, A.L., Coddington, C.C., Stewart, E.A., 2014. *Characteristics indicating adenomyosis coexisting with leiomyomas: a case–control study.* // Hum Reprod, 25(5): Pp. 1177–1182.

[14] Truskinovsky, A.M., Lifschitz-Mercer, B., Czernobilsky, B., 2014. *Hyperplasia and carcinoma in secretory endometrium: a diagnostic challenge.//* Gynecological Pathology, 33(2): Pp. 107-113.

[15] Yang, T.-L., Guo, Y., Li, S.M., Li, S.K., Tian, Q., Liu, Y.-J., Deng, H.-W., 2013. *Ethnic differentiation of copy number variation on chromosome 16p12.3 for association with obesity phenotypes in European and Chinese populations.* // J. Obes (Lond), 37(2): Pp. 188–190.